GENETIC ALTERATIONS OF THE *rII B* CISTRON POLYPEPTIDE OF PHAGE T4[1]

WILLIAM H. McCLAIN[2] AND SEWELL P. CHAMPE

Department of Biological Sciences, Purdue University, Lafayette, Indiana 47907
and
Institute of Microbiology, Rutgers University, The State University of New Jersey, New Brunswick, New Jersey 08903

Received June 9, 1970

ALTHOUGH genetic studies with the *rII* mutants of bacteriophage T4 have provided many insights into genetic mechanisms (BENZER 1959, 1961; CRICK, BARNETT, BRENNER and WATTS-TOBIN 1961; CHAMPE and BENZER 1962a), this system has not been useful for studying gene-protein relationships because of the inability to isolate the rII protein. Recently, however, we described the detection of a peptide, released by trypsin digestion of the total protein from T4-infected cells, which was identified as a fragment of the *rII B* cistron polypeptide (McCLAIN and CHAMPE 1967). By determining the presence or absence of this tryptic peptide in extracts prepared from cells infected with various *B* cistron mutants, it was concluded that the fragment was derived from the middle one-third of the B polypeptide.

The ability to detect this tryptic fragment provides an assay for the intact *B* cistron polypeptide and thus offers a means for its purification. The present paper describes progress in this direction. Although the purification thus far achieved is far from complete, it has been possible to detect certain mutational changes in the polypeptide which directly confirm previous conclusions derived from indirect genetic studies with the *rII* system.

MATERIALS AND METHODS

Escherichia coli strains: Strains B and BB were used as permissive hosts and strain KB as the general nonpermissive host for *rII* mutants (BENZER and CHAMPE 1961). Strains T_3A and its amber suppressorless derivative, T_3A-*su*$^-$, were used for experiments in which proteins were labeled with tryptophan (McCLAIN and CHAMPE 1967). Other *rII* nonpermissive hosts, C600, CA165, and CAJ64, which carry suppressors specific for amber, ochre, and opal (UGA) mutations, respectively, were used for selecting *rII* derivatives (BRENNER and BECKWITH 1966; SAMBROOK, FAN and BRENNER 1967).

Phage strains: All of the *rII* mutants used in this work were derived from the standard type T4B and have been described previously (CHAMPE and BENZER 1962; BRENNER, STRETTON and KAPLAN 1965).

[1] This research was supported by Grant GB-1897 from the National Science Foundation and by Grant GM-10477 from the U.S. Public Health Service. During part of the course of this work, one of us (W.H.M.) received support from Grant GM-779-08, Division of General Medical Sciences, National Institutes of Health.

[2] Present address: Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge, England. Requests for reprints should be addressed to Dr. S. P. CHAMPE at Rutgers University.

Spontaneous revertants of *rII* mutants were selected from KB plates after incubation overnight at 30°C. The independent origin of each revertant was guaranteed by isolating not more than one revertant from an *r*-type plaque picked from a plate seeded with strain B.

Amber (UAG) and opal (UGA) derivatives of an ochre (UAA) mutant were isolated by the methods of SAMBROOK, FAN and BRENNER (1967). The ochre derivative of an opal mutant was isolated using the ochre suppressor strain (CA165) which does not suppress opal mutants (BRENNER, BARNETT, KATZ and CRICK 1967).

Our conditions of labeling phage proteins depend on the cessation of late phage-specific protein synthesis observed in *rII* infected λ lysogens (see MCCLAIN and CHAMPE 1967). When we wished to examine proteins synthesized by a phage derivative having a mutationally altered but functional *B* cistron (e.g., a false revertant), the *A* cistron was inactivated by introducing the deletion mutation *rB45*. In this way we guaranteed that the protein synthesized by all phage-infected cultures would be directly comparable. The desired strain having the inactivated *A* cistron was constructed by recombination with the double mutant *rB45, r638*. Since *r638* is a deletion mutation of the entire *B* cistron, the desired recombinant was identified as giving *r*+ recombinants with *r638* but not with *rB45*.

UV crosses: Ultraviolet light was used in some crosses to stimulate recombination following the procedure of BRENNER, BARNETT, KATZ and CRICK (1967).

Preparation and analysis of tryptic digests of infected cell extracts: Cells of the host strain T_3A (or T_3A-*su*⁻) infected with T4 *rII* mutants or derivatives were labeled with either L-tryptophan-3-^{14}C or L-tryptophan-^{3}H from three to 17 minutes after infection at 37°C. Unfractionated or partially purified protein extracts of these cells were digested with trypsin and the resulting peptide mixture was analyzed by chromatography on Technicon Peptide resin. The details of the labeling, extraction, digestion and chromatography have been given elsewhere (MCCLAIN and CHAMPE 1967).

Fractional precipitation of infected cell extracts: Two 200 ml cultures of *rII* mutant-infected cells, one labeled with ^{14}C-tryptophan and the other labeled with ^{3}H-tryptophan, were chilled, mixed, and centrifuged at 17 min after infection. The cell pellet was resuspended in 10 ml of 0.02 M Tris-HCl buffer, pH 7.2 containing 10^{-3} M EDTA and disrupted in a French press with about one gram uninfected *E. coli* added as carrier. After removal of debris by centrifugation, an equal volume of 0.25% protamine sulfate (Calbiochem) was added to the supernatant with constant stirring, and the resulting precipitate was centrifuged. The pellet was washed by centrifugation, first with 10 ml of 0.1 M KH_2PO_4–0.01 M EDTA, pH 7.2, and then with 3 ml of 0.5 M KH_2PO_4–0.1 M EDTA, pH 7.2. The second supernatant fraction was then precipitated with increasing concentrations of ammonium sulfate in 0.02 M Tris-HCl buffer, pH 7.2 containing 10^{-3} M EDTA. The protein precipitating between 30–40% saturation was richest in component 30 (see RESULTS) and is the fraction selected for further purification on DEAE-cellulose. Throughout all steps of the fractionation, the temperature was maintained at 0–4°C, all buffers contained 5×10^{-4} M dithiothreitol, and centrifugations were at 20,000 × *g* for 15 min.

Chromatography on DEAE-cellulose: The protein precipitated by ammonium sulfate (in the procedure given above) was removed by centrifugation and subsequently resuspended in 5.0 ml of 0.01 M KH_2PO_4, pH. 7.5, and dialyzed against 1 liter of the same buffer at 4°C for 15 hr. After removing any insoluble material by centrifugation, a sample (between 1 and 2 ml) was applied to a 0.9 × 30 cm column of DEAE-cellulose (Whatman Microgranular DE-52) which had been equilibrated with 0.01 M KH_2PO_4, pH 7.5 buffer. After washing with 100 ml of the same buffer, elution of the protein was accomplished by a 200 ml linear gradient of KCl from 0.0 M to 0.5 M in the same buffer. The ^{3}H and ^{14}C activities of 2.0 ml fractions were assayed by drying 0.2 ml samples on filter paper disks which were then washed with trichloroacetic acid followed by ethanol, according to the method of BOLLUM (1966), and finally counted in a scintillation counter. All buffers contained 10^{-3} M EDTA and 5×10^{-4} M dithiothreitol and all operations were performed at 4°C.

RESULTS

Partial purification of the rII B *cistron polypeptide:* Figure 1a illustrates the

detection of a fragment of the *rII B* cistron polypeptide in a crude extract as previously reported (McCLAIN and CHAMPE 1967). This chromatogram shows the separation of ^{14}C- and ^{3}H-labeled peptides resulting from trypsin digestion of an unfractionated extract of a mixture of two sets of phage infected cells—one infected with an *rII* mutant blocked in the synthesis of the *A* cistron polypeptide and labeled with ^{3}H-tryptophan, and the other infected with an *rII* mutant blocked in the synthesis of the *B* cistron polypeptide and labeled with ^{14}C-tryptophan. The ratio of the two labels is relatively constant throughout the chromatogram except in the region of fraction 125. In this region there is an excess of ^{3}H over ^{14}C indicating that this peak (#30) contains a component derived from the *B* cistron polypeptide. Additional experiments confirmed this conclusion and delineated the location of the tryptic fragment to the middle one-third of the B polypeptide.

The detection of this tryptic fragment provides an assay for the intact B polypeptide and makes possible its purification by fractionating an extract in a stepwise manner and selecting those fractions at each stage which are enriched in component 30.

The three stages of fractionation thus far employed are outlined below and are described in detail in MATERIALS AND METHODS. An initial purification of about 3-fold was accomplished by precipitation with protamine sulfate followed by elution with increasing concentrations of phosphate, selecting the material which remains insoluble in 0.1 M phosphate but which dissolves in 0.5 M phosphate. In the second stage, this selected fraction was then precipitated with increasing concentrations of ammonium sulphate, selecting the material which is soluble at 30% saturation but not at 44% saturation. This second stage gives approximately another 3-fold purification. Figure 1b shows a chromatographic analysis, performed in exactly the same manner as for the unfractionated extract of Figure 1a, of a tryptic digest of this 2-stage purified material. In contrast to Figure 1a, ^{3}H-labeled component 30 is now nearly free of ^{14}C-labeled material. Moreover, the complexity of the early part of the chromatogram has been reduced, thereby revealing two additional components (corresponding to 6 and 10 of Figure 1a) which have a large excess of ^{3}H over ^{14}C.

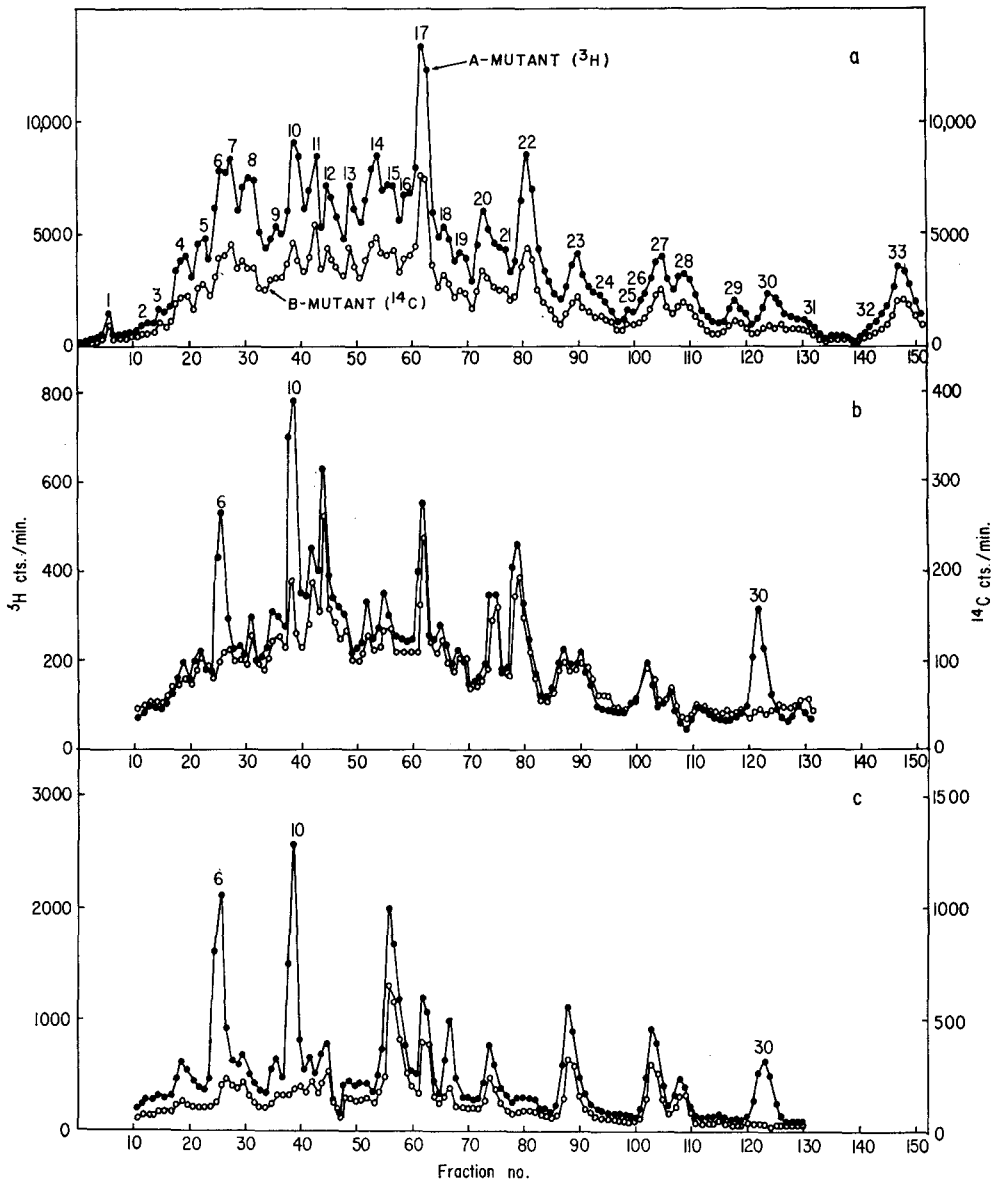
A third stage of purification by chromatography on DEAE-cellulose, selecting the region with the highest $^{3}H/^{14}C$ ratio, affords about another 2-fold enrichment. Figure 1c shows the analysis of the tryptic peptides of the material resulting from this third stage of purification. Again, components 6 and 10 have a great excess of ^{3}H over ^{14}C.

Since components 6 and 10 co-purify with component 30 they are presumably also derived from the *rII* B polypeptide. Although quantitation of components 6 and 10 is often made difficult by variable background components, we nevertheless find, as expected for a common precursor, that the relative amount of label of the three components remains constant throughout the purification—namely approximately 1:2:1 in components 6, 10, and 30, respectively.

Though some of the tryptic peptides of the B polypeptide may be insoluble, it is possible that the three components, 6, 10, and 30 in Figure 1c, contain all of the tryptophan residues of the B polypeptide since the background of the

chromatogram is sufficiently low to permit detection of any which are present. At this stage of purification, approximately 20% of the recovered ^{3}H is in components originating from the B polypeptide. Limited mutational studies on the B polypeptide are feasible with this degree of purity.

Genetic identification of tryptophan codons in the rII B *cistron:* The codon for tryptophan is rather unique: unlike most amino acids, tryptophan has only one codon (UGG) and is connected to both the amber (UAG) and opal (UGA)



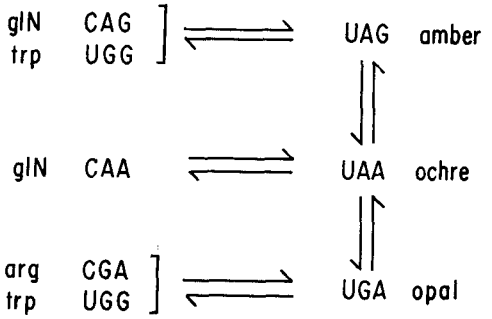


FIGURE 2.—Amino acids related by single-step transition mutations to the amber, ochre, and opal nonsense triplets.

triplets by single-step transition mutations as illustrated in Figure 2. By making use of these relationships, one can predict which mutant sites in the *B* cistron, among the 150-odd which are known, result from mutations in tryptophan codons.

The first line of evidence identifying tryptophan sites is based on the experiments of BRENNER, STRETTON and KAPLAN (1965) which show that hydroxylamine-induced *rII* amber mutations can be divided into two classes on the basis of whether or not the mutation requires DNA replication for expression. An amber mutation in a tryptophan codon would not be expected to require DNA replication for expression of the mutant phenotype, since the hydroxylamine mutated C in the DNA codon would be in the transcribed strand. In contrast, a glutamine amber would require replication before the mutation could be expressed. These experiments identify three presumptive tryptophan amber sites in the *B* cistron: *rHB74*, *rX237*, and *rHB232*.

A second indication of tryptophan sites is the occurrence of a closely linked pair of amber and opal mutations. Such amber–opal pairs are to be expected since both kinds of mutations can occur in the tryptophan codon (UGG), the amber mutation being UGG to UAG and the opal mutation UGG to UGA. In the *B* cistron, three amber–opal pairs have been described: the amber mutations *rHB74*,

FIGURE 1.—Partial purification of the *rII* B polypeptide. The chromatograms show analyses of the peptides resulting from tryptic digestion of the protein from either unfractionated or fractionated extracts prepared from infected cells of two combined cultures: the first infected with an *A* cistron mutant and labeled with ^{3}H-tryptophan and the second infected with a *B* cistron mutant and labeled with ^{14}C-tryptophan. The mutants used were of a type which would be expected to block synthesis of most of the polypeptide product of the respective cistron (either a nonsense mutant whose site is near the N-terminal end of the cistron or a deletion mutant). Thus an excess of ^{3}H over ^{14}C identifies components originating from the B polypeptide. Details of the procedures are given in MATERIALS AND METHODS and by MCCLAIN and CHAMPE (1967).

(a) Digest of unfractionated extract.

(b) Digest of fraction from two-stage fractionation selected for enrichment of component 30.

(c) Digest of fraction from three-stage purification selected for highest $^{3}H/^{14}C$ ratio.

The digests were of nonequivalent aliquots of separate preparations, thus the quantity of label is not comparable from one analysis to another.

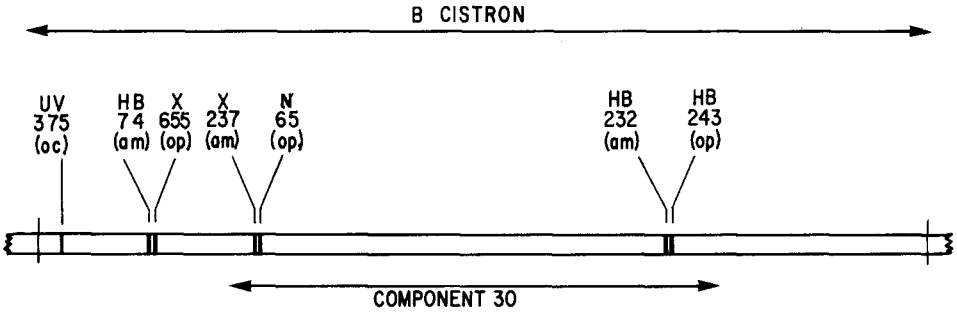


FIGURE 3.—The order of some mutational sites in the *rII B* cistron. The map shows the three known amber–opal pairs in the *rII B* cistron, as discussed in the text, and is drawn approximately to a scale based on the known number of intervening sites (CHAMPE and BENZER 1962a). The region labeled component 30 represents the *maximum* extent of the part of the cistron which codes for component 30 (see Figure 1) as deduced from the experiments described here and reported by MCCLAIN and CHAMPE (1967).

rX237, and *rHB232* are paired, respectively, with the opal mutations *rX655*, *rN65*, and *rHB243* (BRENNER, BARNETT, KATZ and CRICK 1967; KATZ 1968; MCCLAIN 1968).

Thus, the results of the two kinds of experiments are in agreement and make a strong case for predicting the presence and location of three tryptophan sites in the *B* cistron. The relative positions of the sites are given in Figure 3.

The detection of three *B* cistron tryptophan peptides makes possible direct confirmation of the above genetic predictions. We know that amino acid replacements resulting from suppression or reversion of nonsense mutations are confined to the position of the nonsense mutation in the polypeptide chain (BRENNER, STRETTON and KAPLAN 1965; GAREN 1968). Thus, if a particular amber or opal mutation occurred in a tryptophan codon, the corresponding tryptophanyl residue in the polypeptide could be eliminated by a false reversion of the nonsense mutation.

Elimination of component 30 by reversion of an amber mutant: Of the three *B* cistron mutations which are likely to have occurred in a tryptophan codon, the hydroxylamine-induced amber mutation *rHB232* is the best candidate for the site corresponding to component 30 since it is located in the C-terminal end of the region which codes for this component. We tested this prediction by examining spontaneous revertants of *rHB232* for tryptophan-labeled component 30; only two out of nine revertants produced the component. Figure 4 shows a chromatographic comparison between one of the component 30 negative revertants labeled with ^{14}C-tryptophan and the *A* cistron mutant *rB45*, labeled with ^{3}H-tryptophan. This analysis was performed after the 2-stage purification, shown in Figure 1b, in order to allow detection of all three *B* cistron components (6, 10, and 30). The chromatogram shows that the revertant completely lacks component 30 but that 6 and 10 are present in normal or near-normal amounts.

Partial elimination of component 6 by reversion of an opal mutant: Genetic arguments presented above suggest that the opal mutation *rX655* occurred in a

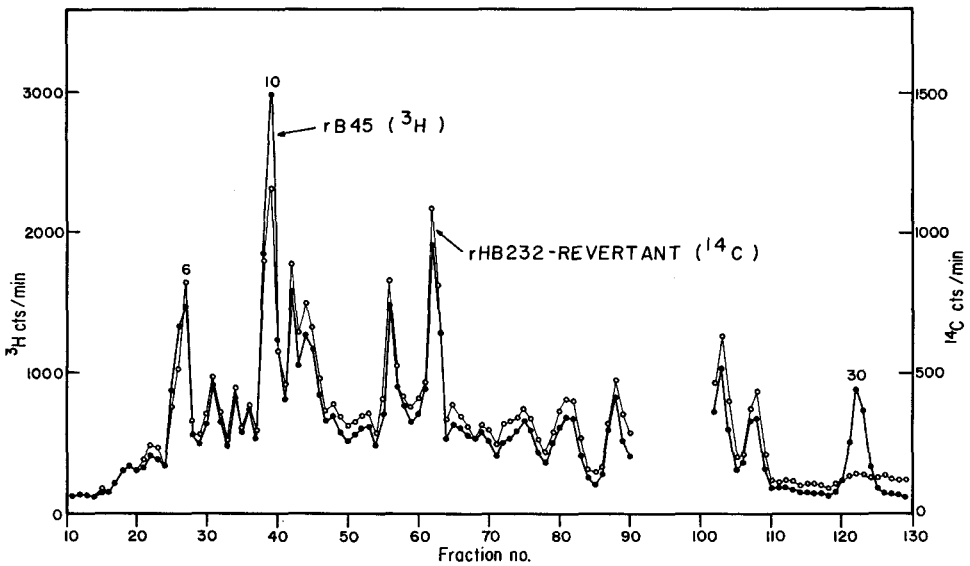


FIGURE 4.—Elimination of component 30 by reversion of *rHB232*. The chromatogram shows the analysis of the peptides labeled with ^{14}C-tryptophan originating from cells infected with an *rHB232* revertant (which also carried the *A* cistron mutation *rB45*, see MATERIALS AND METHODS) as compared to the peptides labeled with ^{3}H-tryptophan originating from cells infected with *rB45*. Digestion of the combined extracts with trypsin was performed after the 2-stage purification illustrated in Figure 1b. The host strain was T_3A-*su*$^-$. The fractions for the missing part of the chromatogram were lost.

tryptophan codon. If so, this tryptophan residue might correspond to that of either component 6 or 10. Since, unlike component 30, components 6 and 10 require purification to be visible, it was desirable to avoid the necessity of screening a sizable number of revertants for one with a non-tryptophan replacement. We accomplished this by first converting *rX655* to its ochre derivative and from this strain isolated a revertant with a wild phenotype. The amino acid substitution of the revertant is unlikely to be tryptophan since this would require two base changes (i.e., UAA to UGG).

Figure 5 shows an analysis of such a revertant performed identically to that of Figure 4. It is seen that the revertant produces normal amounts of components 10 and 30, but component 6 is considerably depressed. The likely reason that component 6 is not completely eliminated by the reversion is that the peak is actually a mixture of peptides, one of *rII* origin and the others of non-*rII* origin, and that the latter peptides are not reproducibly eliminated by the purification method used. Comparisons of several purified preparations show in fact that some components are not always eliminated to the same extent.

Generation of a tryptophan codon: By use of appropriate suppressor hosts it is possible to isolate selectively both the amber (UAG) and opal (UGA) derivatives of an ochre (UAA) mutant (SAMBROOK, FAN and BRENNER 1967). These derivatives, when crossed with each other, should yield only two kinds of

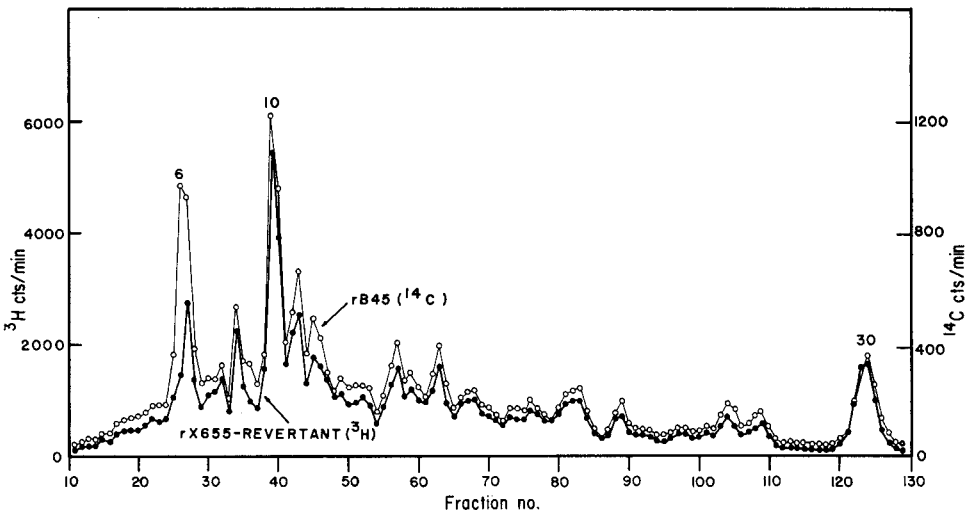


FIGURE 5.—Partial elimination of component 6 by reversion of *rX655*. The chromatogram shows the analysis of the peptides labeled with ^{3}H-tryptophan originating from cells infected with an *rX655* ochre revertant (which also carried the *A* cistron mutation *rB45*, see MATERIALS AND METHODS) as compared to the peptides labeled with ^{14}C-tryptophan originating from cells infected with *rB45*. Digestion of the combined extracts with trypsin was performed after the 2-stage purification illustrated in Figure 1b. The host strain was T_3A-*su*$^-$.

recombinants: one corresponding to the original ochre mutant (UAA) and one which contains the new sequence, UGG. Since the original ochre mutation could not have arisen from a tryptophan codon (UGG) by a single-step mutation, the UGG recombinant should contain an additional tryptophan. These properties of nonsense mutations provide the basis for constructing a phage in which a tryptophan codon has been generated at a particular position in a cistron.

We chose the ochre mutation *rUV375* to test these predictions. Since this mutation affects the left tip of the *B* cistron, a region known to be nonessential for gene activity (CHAMPE and BENZER 1962b), an amino acid replacement at this position would not be expected to have a deleterious effect on cistron activity; hence, the desired recombinant should have a wild phenotype.

Amber and opal derivatives of *rUV375* were isolated and crossed with each other using UV light to stimulate recombination. A 50-fold increase in the frequency of plaques found on KB for the mixed infection, relative to controls, showed that the desired recombinant had been produced. A presumptive UGG recombinant was picked and the tryptophan peptides were examined by the techniques described above. Partial purification was performed before tryptic digestion to aid in detecting the expected new peptide unique to the recombinant. The results are given in Figure 6 where again the *A* cistron mutant *rB45* serves as the control. It is seen that there is a component at fraction 59 which is present only for the recombinant. This experiment has been repeated with the isotopes reversed and the same result was obtained.

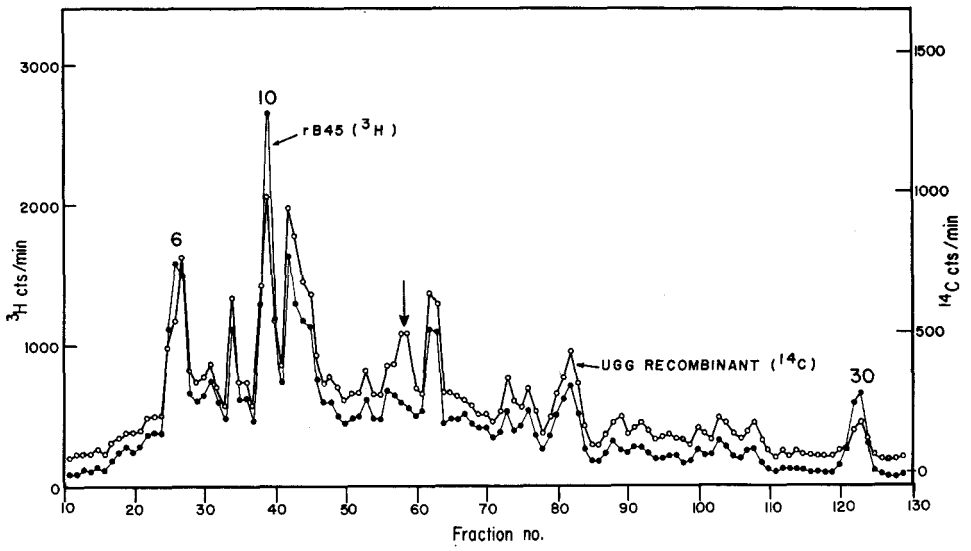


FIGURE 6.—Detection of a tryptophan-containing peptide unique to an amber × opal recombinant. The recombinant with $r+$ phenotype was isolated from the progeny of a cross between the amber and opal derivatives of the ochre mutant *rUV375*. The chromatogram shows the analysis of the peptides labeled with ^{14}C-tryptophan originating from cells infected with the recombinant (which also carried the *A* cistron mutation *rB45*, see MATERIALS AND METHODS) as compared to the peptides labeled with ^{3}H-tryptophan originating from cells infected with *rB45*. Digestion of the combined extracts with trypsin was performed after the 2-stage purification illustrated in Figure 1b. The host strain was T_3A-*su*$^-$. The arrow indicates the position of the new component.

DISCUSSION

Tryptic digestion of tryptophan labeled protein from T4-infected cells produces a peptide (designated component 30) which, as previously described (McCLAIN and CHAMPE 1967), can be identified as a fragment of the polypeptide product of the *rII B* cistron. In the present report we have confirmed the origin of this fragment by showing that it can be used as an assay to purify the intact *rII* B polypeptide. Analysis of partially purified extracts reveals two additional tryptophan-containing components which co-purify with component 30. The constant relative yields of the three components throughout the purification suggests that the precursor of all three components is the *rII B* polypeptide.

The three *B* cistron components in Figure 1c represent about 20% of the recovered label. This stage of purification represents a 20-fold enrichment for component 30 over that found in crude extract. Hence, we estimate that the B polypeptide comprises some 1% of the tryptophan label in an unfractionated extract under our conditions of labeling. Whether this value is an accurate reflection of the fraction of "early" protein which is B polypeptide depends upon the relative abundance of tryptophan among these proteins.

The analysis of tryptic peptides from an unfractionated extract shown in Figure 1a and other similar analyses reveal no indication of a fragment originat-

ing from the *A* cistron polypeptide even though genetic arguments also suggest three tryptophan codons in this cistron (BRENNER, STRETTON and KAPLAN 1965; SCHWARTZ and BRYSON 1969). In the fractionated extracts we would not necessarily expect to find the A polypeptide even if A and B polypeptides are normally complexed since, in this case, the phage contributing component 30 was either an *A* cistron nonsense mutant or a deletion mutant. It should be possible, however, to determine whether the normal A and B polypeptides are physically associated to form an "rII protein" by using a phage with intact *A* and *B* cistrons. In this case enrichment for the B polypeptide during purification should also enrich for the A polypeptide.

Genetic arguments based on the occurrence of paired amber-opal mutational sites and hydroxylamine mutagenesis suggest which of the many mutational sites of the *rII B* cistron correspond to tryptophan codons. By virtue of partial purification of the B polypeptide, it has been possible to confirm some of these predictions. In particular, it has been shown that revertants of the amber mutant *rHB232* and the opal mutant *rX655* have specifically lost components 30 and 6, respectively, consistent with the genetic evidence that these mutations occurred in tryptophan codons. It is presumed, though not yet proved, that the disappearance of these components in the false revertants reflects the loss of their label by conversion of tryptophan to some other amino acid. Alternatively, the false reversion could merely have changed the chromatographic behavior of the peptide without eliminating the tryptophan. The degree of purification, however, is such that any soluble peptide should have been detected wherever it elutes. It is significant in this respect that of twenty false revertants of other amber and ochre mutants (all of which we predicted to have occurred in a glutamine codon) at six different sites near *rHB232*, none was found to eliminate or alter the mobility of component 30.

The third *B* cistron mutation which genetic arguments suggest occurred in a tryptophan codon is *rN65*. So far it has not been possible to demonstrate a correspondence between the presumed tryptophan at this site and component 10, the remaining tryptophan peptide of *B* cistron origin. This may be due to the occurrence of more than one tryptophanyl residue in component 10 which would make the elimination of a single residue difficult to detect.

The tryptophan codon is generated in one of the recombinants from an intracodon recombination between amber and opal mutations affecting the same triplet. Provided these mutations did not originally occur in a tryptophan codon (a condition which can be assured by deriving the amber and opal mutants from an ochre mutant), the recombinant should contain an additional tryptophan in the corresponding polypeptide. This prediction was tested and, as expected, a tryptophan component unique to the recombinant was found. Though this result must be confirmed by other means, it suggests the possibility of placing a tryptophan residue at other sites in the B polypeptide and thus tagging and isolating almost any desired region of the molecule. The technique requires only the presence of a nonleaky ochre or opal mutation in the desired region and that tryptophan be an acceptable missense replacement. One region of the *B* cistron where

this method might be usefully applied is in the neighborhood of the mutational hot spot *r117*, a site at which more than half of all spontaneous *B* cistron mutations occur (BENZER 1961). Knowledge of the amino acid sequence of this region of the B polypeptide might allow the singularity in nucleotide sequence responsible for this extreme mutational instability to be determined.

We are indebted to Dr. IRWIN TESSMAN for his inspiration and valuable suggestions throughout the course of this work.

SUMMARY

The partial purification of the *rII B* cistron polypeptide of bacteriophage T4 is described. Genetic arguments identifying certain mutations in the *B* cistron with tryptophan codons are confirmed directly by analysis of the partially purified *B* cistron polypeptide. A method is described and tested for generating a tryptophan codon at certain selected sites in the *B* cistron.

LITERATURE CITED

BENZER, S., 1959 On the topology of the genetic fine structure. Proc. Natl. Acad. Sci. U.S. **45**: 1607–1620. ——, 1961 On the topography of the genetic fine structure. Proc. Natl. Acad. Sci. U.S. **47**: 403–415.

BENZER, S. and S. P. CHAMPE, 1961 Ambivalent *rII* mutants of phage T4. Proc. Natl. Acad. Sci. U. S. **47**: 1025–1038.

BOLLUM, F. J., 1966 Filter paper disk techniques for assaying radioactive molecules. pp. 296–300. In: *Procedures in Nucleic Acid Research.* Edited by G. L. CANTONI and D. R. DAVIES. Harper and Row, New York.

BRENNER, S., L. BARNETT, E. R. KATZ and F. H. C. CRICK, 1967 UGA: a third nonsense triplet in the genetic code. Nature **213**: 449–450.

BRENNER, S. and J. R. BECKWITH, 1966 Ochre mutants, a new class of suppressible nonsense mutants. J. Mol. Biol. **13**: 629–637.

BRENNER, S., A. O. W. STRETTON and S. KAPLAN, 1965 Genetic code: the 'nonsense' triplets for chain termination and their suppression. Nature **206**: 994–998.

CHAMPE, S. P. and S. BENZER, 1962a. Reversal of mutant phenotypes by 5-fluorouracil: an approach to nucleotide sequence in messenger-RNA. Proc. Natl. Acad. Sci. U. S. **48**: 532–546. ——, 1962b An active cistron fragment. J. Mol. Biol. **4**: 288–292.

CRICK, F. H. C., L. BARNETT, S. BRENNER and R. J. WATTS-TOBIN, 1961 General nature of the genetic code for proteins. Nature **192**: 1227–1232.

GAREN, A., 1968 Sense and nonsense in the genetic code. Science **160**: 149–159.

KATZ, E. R., 1968 Genetic studies on the *rII* gene of bacteriophage T4. Ph.D. thesis, University of Cambridge, U. K.

MCCLAIN, W. H., 1968 Detection of the *rII* B polypeptide of phage T4. Ph.D. thesis, Purdue University, U. S. A.

MCCLAIN, W. H. and S. P. CHAMPE, 1967 Detection of a peptide determined by the *rII B* cistron of phage T4. Proc. Natl. Acad. Sci. U. S. **58**: 1182–1188.

SAMBROOK, J. F., D. P. FAN and S. BRENNER, 1967 A strong suppressor specific for UGA. Nature **214**: 452–453.

SCHWARTZ, R. D. and V. BRYSON, 1969 Nonsense mutants in the *rII A* cistron of bacteriophage T4. J. Virology **4**: 811–815.